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<p>(21) International Application Number: PCT/EP96/03025 (22) International Filing Date: 11 July 1996 (11.07.96) (30) Priority Data: 9514465.5 14 July 1995 (14.07.95) GB (71) Applicant (for all designated States except US): LABORATOIRE GLAXO WELLCOME S.A. [FR/FR]; 43, rue Vineuse, F-75116 Paris (FR). (72) Inventors; and (75) Inventors/Applicants (for US only): DAUGAN, Alain, Claude-Marie [FR/FR]; Laboratoire Glaxo Wellcome S.A., Centre de Recherches, Z.A. de Courtaboeuf, 25, avenue de Québec, F-91940 Les Ulis (FR). GELLIBERT, Françoise [FR/FR]; Laboratoire Glaxo Wellcome S.A., Centre de Recherches, Z.A. de Courtaboeuf, 25, avenue de Québec, F-91940 Les Ulis (FR). (74) Agents: FILLER, Wendy, Anne et al.; Glaxo Wellcome plc, Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report.</p>
<p>(54) Title: CYCLIC GMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS</p> <div style="display: flex; justify-content: space-around; align-items: center;"> <div data-bbox="276 1176 812 1365"> <p style="text-align: center;">(I)</p> </div> <div data-bbox="1055 1176 1266 1344"> <p style="text-align: center;">(1)</p> </div> </div> <p>(57) Abstract</p> <p>A compound of formula (I) and solvates thereof, in which R⁰ represents hydrogen, halogen or C₁₋₆alkyl; R¹ represents hydrogen or C₁₋₆alkyl; R² represents the bicyclic ring (1) which may be optionally substituted by one or more groups selected from halogen and C₁₋₃alkyl; and R³ represents hydrogen or C₁₋₃alkyl. A compound of the present invention is a potent and selective inhibitor of cGMP specific PDE having a utility in a variety of therapeutic areas where such inhibition is beneficial.</p>		

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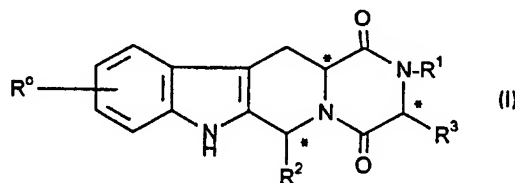
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CYCLIC GMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS

This invention relates to a series of tetracyclic derivatives, to processes for their preparation, pharmaceutical compositions containing them, and their use as therapeutic agents. In particular, the invention relates to tetracyclic derivatives which are potent and selective inhibitors of cyclic guanosine 3',5'-monophosphate specific phosphodiesterase (cGMP specific PDE) having utility in a variety of therapeutic areas where such inhibition is thought to be beneficial, including the treatment of cardiovascular disorders.

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Thus, according to a first aspect, the present invention provides compounds of formula (I)



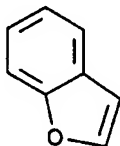
and solvates (e.g. hydrates) thereof, in which:

15

R^0 represents hydrogen, halogen or C_{1-6} alkyl;

R^1 represents hydrogen or C_{1-6} alkyl;

R^2 represents the bicyclic ring



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which may be optionally substituted by one or more groups selected from halogen and C_{1-3} alkyl;

and

R^3 represents hydrogen or C_{1-3} alkyl.

The term "halogen" as used herein denotes bromine, chlorine, fluorine and iodine.

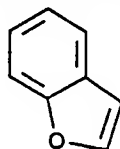
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The terms " C_{1-3} alkyl" and " C_{1-6} alkyl" as used herein denote a straight or branched alkyl chain such as methyl, ethyl, i-propyl, n-butyl, pentyl, hexyl or the like.

A particularly preferred subgroup of compounds according to the present invention are compounds wherein R^0 represents hydrogen.

A further preferred subgroup includes compounds wherein R¹ is selected from hydrogen, methyl and iso-propyl.

Preferably, R² represents the unsubstituted bicyclic ring



5 A still further subgroup of compounds of formula (I), are compounds wherein R³ represents hydrogen or methyl.

It is to be understood that the present invention covers all appropriate combinations of particular and preferred groupings hereinabove.

10 The compounds of formula (I) may contain one or more asymmetric centres and thus can exist as enantiomers or diastereoisomers. It is to be understood that the invention includes both mixtures and separate individual isomers of the compounds of formula (I). Particularly preferred are 6R and 12aR isomers.

Particular individual compounds of the invention include:

15 (6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2-methyl-pyrazino [2',1':6,1]pyrido[3,4-b]indole-1,4-dione;
(6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-pyrazino[2',1':6,1] pyrido [3,4-b]indole-1,4-dione;
(3S, 6R, 12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-3-methyl-pyrazino[2',1':6,1] pyrido [3,4-b]indole-1,4-dione;
20 (3S, 6R, 12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2,3-dimethyl-pyrazino[2',1':6,1] pyrido [3,4-b]indole-1,4-dione;
(6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2-isopropyl-pyrazino [2',1':6,1] pyrido [3,4-b]indole-1,4-dione;
and physiologically acceptable solvates (e.g. hydrates) thereof.

25 A most particular compound of the invention is
(6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2-methyl-pyrazino [2',1':6,1]pyrido[3,4-b]indole-1,4-dione;
and physiologically acceptable solvates (e.g. hydrates) thereof.

30 It has been shown that compounds of the present invention are potent and selective inhibitors of cGMP specific PDE. Thus, compounds of formula (I) are

of interest for use in therapy, specifically for the treatment of a variety of conditions where inhibition of cGMP specific PDE is thought to be beneficial.

As a consequence of the selective PDE 5 inhibition exhibited by compounds of the present invention, cGMP levels are elevated, which in turn can give rise to beneficial anti-platelet, anti-neutrophil, anti-vasospastic, vasodilatory, natriuretic and diuretic activities as well as potentiation of the effects of endothelium-derived relaxing factor (EDRF), nitrovasodilators, atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP) and endothelium-dependent relaxing agents such as bradykinin, acetylcholine and 5-HT₁. The compounds of formula (I) therefore have utility in the treatment of a number of disorders, including stable, unstable and variant (Prinzmetal) angina, hypertension, pulmonary hypertension, congestive heart failure, renal failure, atherosclerosis, conditions of reduced blood vessel patency (e.g. post-percutaneous transluminal coronary angioplasty), peripheral vascular disease, vascular disorders such as Raynaud's disease, inflammatory diseases, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, glaucoma, erectile dysfunction and diseases characterised by disorders of gut motility (e.g. irritable bowel syndrome).

It will be appreciated that references herein to treatment extend to prophylaxis as well as treatment of established conditions.

It will also be appreciated that 'a compound of formula (I),' or a physiologically acceptable salt or solvate thereof can be administered as the raw compound, or as a pharmaceutical composition containing either entity.

There is thus provided as a further aspect of the invention a compound of formula (I) for use in the treatment of stable, unstable and variant (Prinzmetal) angina, hypertension, pulmonary hypertension, chronic obstructive pulmonary disease, congestive heart failure, renal failure, atherosclerosis, conditions of reduced blood vessel patency, (e.g. post-PTCA), peripheral vascular disease, vascular disorders such as Raynaud's disease, inflammatory diseases, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, glaucoma, erectile dysfunction or diseases characterised by disorders of gut motility (e.g. IBS).

According to another aspect of the invention, there is provided the use of a compound of formula (I) for the manufacture of a medicament for the treatment of stable, unstable and variant (Prinzmetal) angina, hypertension, pulmonary hypertension, chronic obstructive pulmonary disease, congestive heart failure,

renal failure, atherosclerosis, conditions of reduced blood vessel patency, (e.g. post-PTCA), peripheral vascular disease, vascular disorders such as Raynaud's disease, inflammatory diseases, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, glaucoma, erectile dysfunction or diseases characterised by disorders of gut motility (e.g. IBS).

In a further aspect, the invention provides a method of treating stable, unstable and variant (Prinzmetal) angina, hypertension, pulmonary hypertension, chronic obstructive pulmonary disease, congestive heart failure, renal failure, atherosclerosis, conditions of reduced blood vessel patency, (e.g. post-PTCA), peripheral vascular disease, vascular disorders such as Raynaud's disease, inflammatory diseases, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, glaucoma, erectile dysfunction or diseases characterised by disorders of gut motility (e.g. IBS) in a human or non-human animal body which comprises administering to said body a therapeutically effective amount of a compound with formula (I).

Compounds of the invention may be administered by any suitable route, for example by oral, buccal, sub-lingual, rectal, vaginal, nasal, topical or parenteral (including intravenous, intramuscular, subcutaneous and intracoronary) administration. Oral administration is generally preferred.

For administration to man in the curative or prophylactic treatment of the disorders identified above, oral dosages of a compound of formula (I) will generally be in the range of from 0.5-800mg daily for an average adult patient (70kg). Thus for a typical adult patient, individual tablets or capsules contain from 0.2-400mg of active compound, in a suitable pharmaceutically acceptable vehicle or carrier, for administration in single or multiple doses, once or several times per day. Dosages for intravenous, buccal or sublingual administration will typically be within the range of from 0.1-400 mg per single dose as required. In practice the physician will determine the actual dosing regimen which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case but there can be individual instances in which higher or lower dosage ranges may be merited, and such are within the scope of this invention.

For human use, a compound of the formula (I) can be administered alone, but will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard

pharmaceutical practice. For example, the compound may be administered orally, buccally or sublingually, in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavouring or colouring agents. Such liquid preparations may be prepared with pharmaceutically acceptable additives such as suspending agents (e.g. methylcellulose, a semi-synthetic glyceride such as witepsol or mixtures of glycerides such as a mixture of apricot kernel oil and PEG-6 esters or mixtures of PEG-8 and caprylic/capric glycerides). A compound may also be injected parenterally, for example intravenously, intramuscularly, subcutaneously or intracoronarily. For parenteral administration, the compound is best used in the form of a sterile aqueous solution which may contain other substances, for example salts, or monosaccharides such as mannitol or glucose, to make the solution isotonic with blood.

Thus, the invention provides in a further aspect a pharmaceutical composition comprising a compound of the formula (I) together with a pharmaceutically acceptable diluent or carrier therefor.

There is further provided by the present invention a process of preparing a pharmaceutical composition comprising a compound of formula (I), which process comprises mixing a compound of formula (I) together with a pharmaceutically acceptable diluent or carrier therefor.

A compound of formula (I) may also be used in combination with other therapeutic agents which may be useful in the treatment of the above-mentioned disease states. The invention thus provides, in another aspect, a combination of a compound of formula (I) together with another therapeutically active agent.

The combination referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical compositions comprising a combination as defined above together with a pharmaceutically acceptable diluent or carrier comprise a further aspect of the invention.

The individual components of such a combination may also be administered either sequentially or simultaneously in separate pharmaceutical formulations.

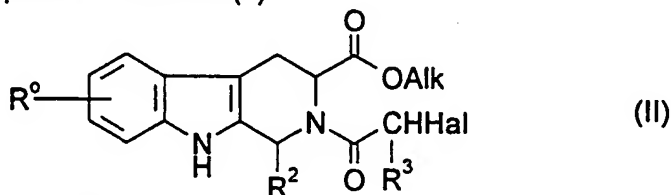
Appropriate doses of known therapeutic agents for use in combination with a compound of formula (I) will be readily appreciated by those skilled in the art.

Compounds of formula (I) may be prepared by any suitable method known in the art or by the following processes which form part of the present invention. In

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the methods below R^0 , R^1 , R^2 and R^3 are as defined in formula (I) above unless otherwise indicated.

Thus, a first process (A) for preparing a compound of formula (I) comprises treating a compound of formula (II)

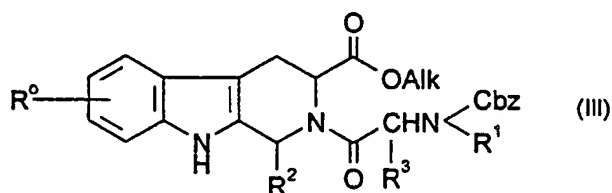


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(in which Alk represents C_{1-6} alkyl, e.g. methyl or ethyl, and Hal is a halogen atom, e.g. chlorine) with a primary amine R^1NH_2 in a suitable solvent such as an alcohol (e.g. methanol or ethanol) or a mixture of solvents, conveniently at a temperature of from 20°C to reflux (e.g. at about 50°C).

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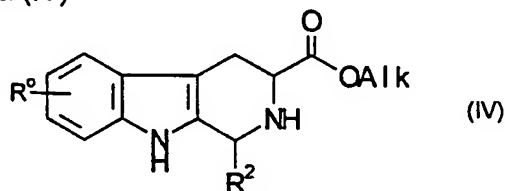
According to a second process (B) for preparing a compound of formula (I) comprises hydrogenating a compound of formula (III)



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in which Alk is defined as above and Cbz represents a carbobenzyloxy group, in the presence of a catalyst e.g. palladium on activated carbon in a suitable solvent such as an alcohol, e.g. methanol or ethanol, at elevated temperature.

A compound of formula (II) may conveniently be prepared by treating a compound of formula (IV)

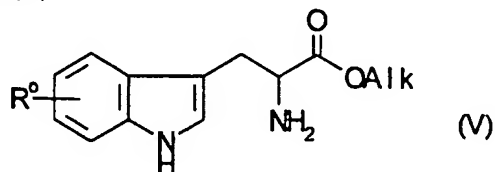


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with a haloacetyl halide (e.g. chloroacetyl chloride) in a suitable solvent such as a halogenated hydrocarbon (e.g. trichloromethane or dichloromethane), or an ether (e.g. tetrahydrofuran), preferably in the presence of a base such as an organic amine (e.g. a trialkylamine such as triethylamine) or an alkali metal

carbonate or bicarbonate (e.g. NaHCO_3). The reaction may conveniently be effected at a temperature of from -20°C to $+20^\circ\text{C}$ (e.g. at about 0°C).

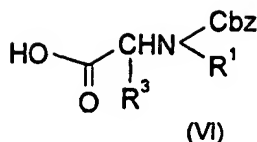
A compound of formula (IV) may conveniently be prepared from a tryptophan alkyl ester of formula (V)



This step comprises a Pictet-Spengler cyclisation between a compound of formula (V) and an aldehyde R^2CHO . The reaction may conveniently be effected in a suitable solvent such as a halogenated hydrocarbon (e.g. dichloromethane) or an aromatic hydrocarbon (e.g. toluene) in the presence of an acid such as trifluoroacetic acid. The reaction may conveniently be carried out at a temperature of from -20°C to reflux to provide a compound of formula (III) in one step. The reaction may also be carried out in a solvent such as an aromatic hydrocarbon (e.g. benzene or toluene) under reflux, optionally using a Dean-Stark apparatus to trap the water produced. The reaction provides a mixture of cis and trans isomers which may be either individual enantiomers or racemates of pairs of cis or trans isomers depending upon whether racemic or enantiomerically pure tryptophan alkyl ester was used as the starting material. Individual cis or trans enantiomers may conveniently be separated from mixtures thereof by fractional crystallisation or by chromatography (e.g. flash column chromatography) using appropriate solvents and eluents. Similarly, pairs of cis and trans isomers may be separated by chromatography (e.g. flash column chromatography) using appropriate eluents. An optically pure trans isomer may also be converted to an optically pure cis isomer using suitable epimerisation procedures. One such procedure comprises treating the trans isomer or a mixture (e.g. 1 : 1 mixture) of cis and trans isomers with methanolic or aqueous hydrogen chloride at a temperature of from 0°C to the refluxing temperature of the solution. The mixture is then subjected to chromatography (e.g. flash column chromatography) to separate the resulting diastereoisomers.

A compound of formula (III) may be prepared by reaction of a compound of formula (IV) as hereinbefore described, with a compound of formula (VI)

8



wherein Cbz is defined above. Suitably, the reaction is carried out in the presence of 1,3-dicyclohexyl carbodiimide (DCC), in a solvent such as halogenated hydrocarbon (e.g. dichloromethane) from 0°C to room temperature.

5 Compounds of formula (V) and (VI) are known compounds or may be prepared by standard methods hereinafter described.

Compounds of the invention may be isolated in association with solvent molecules by crystallisation from or evaporation of an appropriate solvent.

Thus, according to a further aspect of the invention, we provide a process (C) for preparing a compound of formula (I) or a solvate (e.g. hydrate) thereof which comprises process (A) or (B) as hereinbefore described followed by

- i) an interconversion step; and/or either
- ii) solvate (e.g. hydrate) formation.

15 The synthesis of the compounds of the invention and of the intermediates for use therein are illustrated by the following, non-limiting Examples.

Intermediates 1 and 2

20 (1R,3R)-Methyl 1,2,3,4-tetrahydro-1-(5-benzofuranyl)-9H-pyrido[3,4-b]indole-3-carboxylate, cis isomer and
(1S,3R)-methyl 1,2,3,4-tetrahydro-1-(5-benzofuranyl)-9H-pyrido[3,4-b]indole-3-carboxylate trans isomer

To a stirred solution of D-tryptophan methyl ester (3.73g) and 5-formyl-benzofuran¹ (2.5 g) in anhydrous dichloromethane (100 mL) cooled at 0°C was added dropwise trifluoroacetic acid (2.63 mL) and the solution was allowed to react at ambient temperature. After 72 hours, the solution was washed with a saturated aqueous solution of NaHCO₃, then with water and dried over Na₂SO₄. The organic layer was evaporated under reduced pressure and the residue was purified by flash chromatography eluting with dichloromethane / ethyl acetate (90/10) to give first the cis isomer (Intermediate 1) (3 g) as an amorphous compound, followed by the trans isomer (Intermediate 2) (2.5 g) as white crystals, m.p. : 194-195°C.

¹The synthesis of 5-formyl-benzofuran is described in Chimie Thérapeutique 4, pp 221-227 (1966).

5 Intermediate 3

(1R,3R)-Methyl 1,2,3,4-tetrahydro-1-(5-benzofuranyl)-2-chloroacetyl-9H-pyrido[3,4-b]indole-3-carboxylate

To a stirred solution of Intermediate 1 (2 g) and triethylamine (0.88 mL) in anhydrous dichloromethane (40 mL) cooled at 0°C was added dropwise
10 chloroacetylchloride (0.5 mL) and the solution was stirred at the same temperature for 1 hour. The solution was washed with water, dried over Na₂SO₄ and evaporated to dryness and the residue was crystallised from methanol to give the title compound (1.8 g) as pale yellow crystals.
m.p. : 227-228°C.

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Intermediate 4

(1R,3R)-Methyl 1,2,3,4-tetrahydro-1-(5-benzofuranyl)-2-(2-(S)-benzyloxycarbonylaminopropionyl)-9H-pyrido[3,4-b]indole-3-carboxylate

To a stirred solution of (S)-2-benzyloxycarbonylaminopropionic acid (1.3 g) and
20 1,3-dicyclohexyl carbodiimide (DCC) (1.2 g) in anhydrous dichloromethane (50 ml) at 0°C was added Intermediate 1 (1.0 g). The resulting mixture was stirred for 72 hours then the resulting precipitate filtered off. The filtrate was evaporated to dryness and the residue purified by flash chromatography, eluting with cyclohexane/ethyl acetate (60/40) to give the title compound as white
25 crystals (1.4 g)
m.p. : 91-92°C

Intermediate 5

(1R,3R)-Methyl 1,2,3,4-tetrahydro-1-(5-benzofuranyl)-2-[2-(S)-

30 benzyloxycarbonylmethylamino)propionyl]-9H-pyrido[3,4-b]indole-3-carboxylate

The same procedure as employed in the preparation of Intermediate 4 but starting from 2-(S)-benzyloxycarbonylmethylamino)propionic acid (0.82 g) and using Intermediate 1 (0.6 g), DCC (0.72 g) and dichloromethane (25 ml) gave after chromatography, eluting with cyclohexane/ethyl acetate (70/30), the title compound as a white foam.
35

^1H NMR (240MHz, CDCl_3) δ 7.7(s,1H), 7.6(d,2H), 7.4-7.05(m,11H), 6.6(d,1H), 5.4-5.0(m,4H), 3.5(d,1H), 3.0(m,1H), 2.9-2.7(m,6H), 2.6(dd,1H), 1.3(s,3H).

Example 1

5 (6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2-methyl-pyrazino
[2',1':6,1]pyrido[3,4-b]indole-1,4-dione

To a stirred suspension of Intermediate 3 (0.42 g) in methanol (30 mL) was added at ambient temperature a solution of methylamine (33% in EtOH) (0.47 mL) and the resulting mixture was heated at 50°C under N_2 for 72 hours. The solvent was removed under reduced pressure and dissolved in dichloromethane. After washing with water, drying over Na_2SO_4 and evaporating to dryness, the crude product was purified by crystallisation from methanol to give the title compound as white crystals (0.21 g).
m.p. : 291-293°C.

15 Analysis for $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_3$:
Calculated: C,71.68;H,4.97;N,10.90;
Found:C,71.5;H,4.91;N,10.74%.
 $[\alpha]_D^{20} = +55.7^\circ$ (C=1; CHCl_3).

20 The following compounds were obtained in a similar manner:

Example 2

(6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-pyrazino[2',1':6,1]
pyrido [3,4-b]indole-1,4-dione

25 The same procedure as employed in the preparation of Example 1 but starting from ammonia and Intermediate 3 gave, after recrystallisation from methanol, the title compound as white crystals.
m.p. : 310-311°C.

Analysis for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_3$ (0.4 MeOH):
30 Calculated: C,70.03; H,4.88; N,10.94;
Found: C,70.01; H,4.8; N,10.61%;
 $[\alpha]_D^{20} = +60.4^\circ$ (C=0.5;pyridine).

Example 3

(6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2-isopropyl-pyrazino
[2',1':6,1] pyrido [3,4-b]indole-1,4-dione

The same procedure as employed in the preparation of Example 1 but starting from isopropylamine and Intermediate 3 gave, after recrystallisation from methanol, the title compound as white crystals.

m.p. : 291-292°C

Analysis for C₂₅H₂₃N₃O₃ (0.6 MeOH):

Calculated: C,71.06; H,5.92; N,9.71;

Found: C,70.94; H,5.62; N,9.77%.

[α]_D²⁰ = +37.9° (C=1; CHCl₃).

Example 4

(3S, 6R, 12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-3-methyl-
pyrazino[2',1':6,1] pyrido [3,4-b]indole-1,4-dione:

A solution of Intermediate 4 (0.3 g) in the presence of 10%Pd/C (30 mg) in methanol (10 ml) was stirred under an atmosphere of hydrogen at 50 °C for two hours. The reaction mixture was cooled, filtered through Celite, the filter cake washed with methanol and the filtrate evaporated in vacuo. The residue was purified by flash chromatography, eluting with dichloromethane/methanol (98/2) to give the title compound as white crystals after recrystallisation from methanol (0.15 g)

m.p. : 150-151°C

Analysis for C₂₃H₁₉N₃O₃ (0.1MeOH)

Calculated: C,71.39; H,5.03; N,10.81;

Found: C,71.08; H,5.16; N,10.50%;

[α]_D²⁰ = +50° (C=0.25; CHCl₃).

Example 5

(3S, 6R, 12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2,3-dimethyl-
pyrazino[2',1':6,1] pyrido [3,4-b]indole-1,4-dione:

The same procedure as employed in the preparation of Example 4 but starting from Intermediate 5 (0.52 g) and using 10%Pd/C (50 mg) in methanol (20 ml) gave, after recrystallisation from methanol, the title compound as white crystals (40 mg).

m.p. : 323-324°C

Analysis for $C_{24}H_{21}N_3O_3$. (0.1Methanol)

Calculated: C,71.52; H,5.35; N,10.43;

Found: C,71.71; H,5.44; N,10.39%;

$[\alpha]^{20}_D = +53^\circ$ (C=0.35; $CHCl_3$).

5

TABLETS FOR ORAL ADMINISTRATION

A. Direct Compression

1.	mg/tablet
Active ingredient	50.0
Crospovidone USNF	8.0
Magnesium Stearate Ph Eur	1.0
Anhydrous Lactose	141.0

10

The active ingredient was sieved and blended with the excipients. The resultant mix was compressed into tablets.

2.	mg/tablet
Active ingredient	50.0
Colloidal Silicon Dioxide	0.5
Crospovidone	8.0
Sodium Lauryl Sulphate	1.0
Magnesium Stearate Ph Eur	1.0
Microcrystalline Cellulose USNF	139.5

15

The active ingredient was sieved and blended with the excipients. The resultant mix was compressed into tablets.

B. WET GRANULATION

1.	mg/tablet
Active ingredient	50.0
Polyvinyl pyrrolidone	150.0

Polyethylene glycol	50.0
Polysorbate 80	10.0
Magnesium Stearate Ph Eur	2.5
Croscarmellose Sodium	25.0
Colloidal Silicon Dioxide	2.5
Microcrystalline Cellulose USNF	210.0

The polyvinyl pyrrolidone, polyethylene glycol and polysorbate 80 were dissolved in water. The resultant solution was used to granulate the active ingredient. After drying the granules were screened, then extruded at elevated temperatures and pressures. The extrudate was milled and/or screened then was blended with the microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide and magnesium stearate. The resultant mix was compressed into tablets.

2.	mg/tablet
Active ingredient	50.0
Polysorbate 80	3.0
Lactose Ph Eur	178.0
Starch BP	45.0
Pregelatinised Maize Starch BP	22.5
Magnesium Stearate BP	1.5

The active ingredient was sieved and blended with the lactose, starch and pregelatinised maize starch. The polysorbate 80 was dissolved in purified water. Suitable volumes of the polysorbate 80 solution were added and the powders were granulated. After drying, the granules were screened and blended with the magnesium stearate. The granules were then compressed into tablets.

Tablets of other strengths may be prepared by altering the ratio of active ingredient to the other excipients.

FILM COATED TABLETS

The aforementioned tablet formulations were film coated.

Coating Suspension	% w/w
Opadry white†	13.2
Purified water Ph Eur	to 100.0*

* The water did not appear in the final product. The maximum theoretical weight of solids applied during coating was 20mg/tablet.

† Opadry white is a proprietary material obtainable from Colorcon Limited, UK which contains hydroxypropyl methylcellulose, titanium dioxide and triacetin.

The tablets were film coated using the coating suspension in conventional film coating equipment.

CAPSULES

1.	mg/capsule
Active ingredient	50.0
Lactose	148.5
Polyvinyl pyrrolidone	100.0
Magnesium Stearate	1.5

The active ingredient was sieved and blended with the excipients. The mix was filled into size No. 1 hard gelatin capsules using suitable equipment.

2.	mg/capsule
Active ingredient	50.0
Microcrystalline Cellulose	233.5
Sodium Lauryl Sulphate	3.0
Crospovidone	12.0
Magnesium Stearate	1.5

The active ingredient was sieved and blended with the excipients. The mix was filled into size No. 1 hard gelatin capsules using suitable equipment.

- 5 Other doses may be prepared by altering the ratio of active ingredient to excipient, the fill weight and if necessary changing the capsule size.

3.	mg/capsule
Active ingredient	50.0
Labrafil M1944CS	to 1.0 ml

- 10 The active ingredient was sieved and blended with the Labrafil. The suspension was filled into soft gelatin capsules using appropriate equipment.

Inhibitory effect on cGMP-PDE

- 15 cGMP-PDE activity of compounds of the present invention was measured using a one-step assay adapted from Wells et al. (Wells, J. N., Baird, C. E., Wu, Y. J. and Hardman, J. G., Biochim. Biophys. Acta 384, 430 (1975)). The reaction medium contained 50mM Tris-HCl, pH 7.5, 5mM Mg-acetate, 250µg/ml 5'-Nucleotidase, 1mM EGTA and 0.15µM 8-[H³]-cGMP. The enzyme used was a human recombinant PDE V (ICOS, Seattle USA).

- 20 Compounds of the invention were dissolved in DMSO finally present at 2% in the assay. The incubation time was 30 minutes during which the total substrate conversion did not exceed 30%.

- 25 The IC₅₀ values for the compounds examined were determined from concentration-response curves using typically concentrations ranging from 10nM to 10µM. Tests against other PDE enzymes using standard methodology also showed that compounds of the invention are highly selective for the cGMP specific PDE enzyme.

- 30 cGMP level measurements

Rat aortic smooth muscle cells (RSMC) prepared according to Chamley et al. in Cell Tissue Res. 177, 503 - 522 (1977) were used between the 10th and 25th

passage at confluence in 24-well culture dishes. Culture media was aspirated and replaced with PBS (0.5ml) containing the compound tested at the appropriate concentration. After 30 minutes at 37°C, particulates guanylate cyclase was stimulated by addition of ANF (100nM) for 10 minutes. At the end of incubation, the medium was withdrawn and two extractions were performed by addition of 65% ethanol (0.25ml). The two ethanolic extracts were pooled and evaporated until dryness, using a Speed-vac system. c-GMP was measured after acetylation by scintillation proximity immunoassay (AMERSHAM). The EC₅₀ values are expressed as the dose giving half of the stimulation at saturating concentrations

Biological data

The compounds according to the present invention were typically found to exhibit an IC₅₀ value of less than 500 nM and an EC₅₀ value of less than 5 µM. In vitro test data for representative compounds of the invention is given in the following table:

Table 1. *In vitro* results

Example No.	IC ₅₀ nM	EC ₅₀ µM
1	15	0.6
2	20	<1
3	30	<1
4	8	<1
5	8	<1

20

The hypotensive effects of compounds according to the invention as identified in Table 2 were studied in conscious spontaneously hypertensive rats (SHRs). The compounds were administered orally at a dose of 5 mg/kg in a mixture of 5% DMF and 95% olive oil. Blood pressure was measured from a catheter inserted in the carotid artery and recorded for 5 hours after administration. The results are expressed as Area Under the Curve (AUC from 0 to 5 hours, mmHg.hour) of the fall in blood pressure over time.

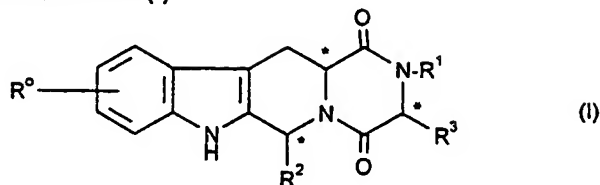
25

Table 2. *In vivo* results

Example No.	AUC PO (mmHg.h)
1	137
2	93
3	108
4	101
5	89

CLAIMS

1. A compound of formula (I)

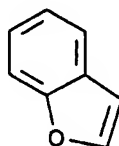


and solvates thereof, in which:

R⁰ represents hydrogen, halogen or C₁₋₆ alkyl;

R¹ represents hydrogen or C₁₋₆alkyl;

R² represents the bicyclic ring



which may be optionally substituted by one or more groups selected from halogen and C₁₋₃alkyl;

and

R³ represents hydrogen or C₁₋₃alkyl.

2. A compound according to Claim 1 wherein R⁰ represents hydrogen.

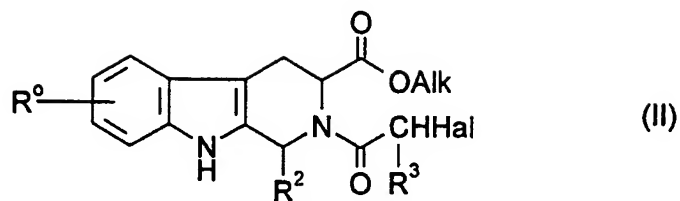
3. A compound according to Claim 1 or Claim 2 wherein R¹ is selected from hydrogen, methyl, and iso-propyl.

4. A compound according to any of Claims 1-3 wherein R³ represents hydrogen or methyl.

5. (6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2-methyl-pyrazino [2',1':6,1]pyrido[3,4-b]indole-1,4-dione;
 (6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-pyrazino[2',1':6,1] pyrido [3,4-b]indole-1,4-dione;
 (3S, 6R, 12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-3-methyl-pyrazino[2',1':6,1] pyrido [3,4-b]indole-1,4-dione;

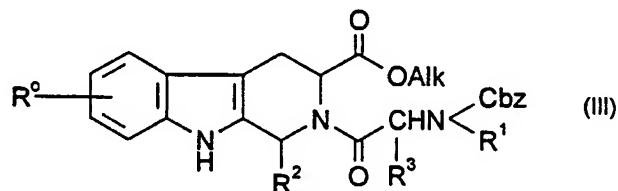
- (3S, 6R, 12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2,3-dimethyl-pyrazino[2',1':6,1] pyrido [3,4-b]indole-1,4-dione;
(6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2-isopropyl-pyrazino
[2',1':6,1] pyrido [3,4-b]indole-1,4-dione;
5 and physiologically acceptable solvates thereof.
6. (6R,12aR)-2,3,6,7,12,12a-Hexahydro-6-(5-benzofuranyl)-2-methyl-pyrazino
[2',1':6,1]pyrido[3,4-b]indole-1,4-dione.
and physiologically acceptable solvates thereof.
10
7. A compound according to any of Claims 1-6 for use in therapy.
8. A compound according to any of Claims 1-6 for use in the treatment of
conditions where inhibition of cGMP specific PDE is of therapeutic benefit.
15
9. Use of a compound according to any of Claims 1-6 for the manufacture of a
medicament for the treatment of conditions where inhibition of cGMP specific
PDE is of therapeutic benefit.
- 20 10. A method of treating conditions where inhibition of cGMP specific PDE is of
therapeutic benefit, in a human or non-human animal body, which comprises
administering to said body a therapeutically effective amount of a compound
according to any one of Claims 1-6.
- 25 11. A pharmaceutical composition comprising a compound according to any of
Claims 1-6, together with a pharmaceutically acceptable diluent or carrier
therefor.
- 30 12. A process of preparing a pharmaceutical composition comprising a
compound according to any of Claims 1-6, which process comprises mixing said
compound together with a pharmaceutically acceptable diluent or carrier
therefor.
- 35 13. A process (A) for preparing a compound according to Claim 1 comprising
treating a compound of formula (II)

20



in which Alk represents C₁₋₆alkyl, and Hal is a halogen atom, with a primary amine R¹NH₂; or,
a process (B) comprising hydrogenating a compound of formula (III)

5



in which Alk is defined as above and Cbz represents a carbobenzyloxy group, in the presence of a catalyst in a suitable solvent such as an alcohol, at elevated temperature: or

10 a process (C) which comprises process (A) or (B) followed by

- i) an interconversion step; and/or either
- iii) solvate formation.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/03025

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D471/14 A61K31/395 //(C07D471/14,241:00,221:00,209:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 05661 (PFIZER) 17 March 1994 see abstract ---	1-12
A	GB,A,1 454 171 (COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH) 27 October 1976 see claims ---	1,7-11
P,X	WO,A,95 19978 (GLAXO) 27 July 1995 see examples 36,37,91,92 -----	1-13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

22 October 1996

Date of mailing of the international search report

31.10.96

Name and mailing address of the ISA

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Authorized officer

De Jong, B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 96/03025

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 10 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 96/03025

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9405661	17-03-94	CA-A- 2138298	17-03-94
		EP-A- 0656898	14-06-95
		FI-A- 950889	27-02-95
		JP-T- 7506838	27-07-95

GB-A-1454171	27-10-76	NONE	

WO-A-9519978	27-07-95	AU-A- 1574895	08-08-95
		CA-A- 2181377	27-07-95
		FI-A- 962927	19-07-96
		NO-A- 963015	09-09-96
		ZA-A- 9500424	27-09-95
